



# Magnetic Resonance Imaging of Transplanted Mouse Islets Labeled With Chitosan-Coated Superparamagnetic Iron Oxide Nanoparticles

J.-H. Juang, J.-J. Wang, C.-R. Shen, C.-H. Kuo, Y.-W. Chien, H.-Y. Kuo, Z.-T. Tsai, and T.-C. Yen

## ABSTRACT

Although only 10% of islet recipients maintain insulin independence, 80% of them are C-peptide positive at 5 years after transplantation. To better understand the fate of transplanted islets, a magnetic resonance imaging (MRI) technique has been used to detect Feridex-labeled islet grafts in rodents. In this study, we used a novel MRI contrast agent, chitosan-coated superparamagnetic iron oxide (CSPIO) nanoparticles, to monitor mouse islet grafts. Male inbred C57BL/6 mice were used as donors and recipients of islet transplantation. The islet cytotoxicity was evaluated by fluorescein diacetate and propidium iodide staining for RAW cells incubated with CSPIO. After being incubated overnight with and without CSPIO (10 mg/mL), 300 islets were transplanted under the left kidney capsule of each mouse. After transplantation, 3.0-Tesla MRI of the recipients was performed biweekly until 19 weeks. At the end of study, the islet graft was removed for insulin and Prussian blue staining. The cell death rates in RAW cells did not increase with increasing CSPIO concentrations or incubation time. The grafts of CSPIO-labeled islets were visualized on MRI scans as distinct hypointense spots homogeneously located at the upper pole of left kidney. Their MRI signal was 30%–50% that of control islets and was maintained throughout the follow-up period. At 18 weeks, the histology of CSPIO-labeled islet graft revealed the insulin- and iron-stained areas to be almost identical. Our results indicate that isolated mouse islets labeled with CSPIO nanoparticles can be effectively and safely imaged by using MRI as long as 18 weeks after transplantation.

Recently, the Edmonton Protocol has markedly improved the success rate of human islet transplantation.<sup>1</sup> However,  $\geq 2$  pancreases are usually required to achieve normoglycemia. Moreover, long-term function of the transplanted islets has been disappointing.<sup>2,3</sup> Allograft failure may be due to nonimmunologic (eg, insufficient  $\beta$ -cell mass and islet engraftment problems) as well as immunologic (eg, immune rejection, toxicity of immunosuppressants and autoimmune recurrence) factors.<sup>4</sup> Although only 10% of recipients maintain insulin independence, 80% of them are C-peptide positive at 5 years after islet transplantation.<sup>2</sup> To better understand the fate of transplanted islets, a magnetic resonance imaging (MRI) technique has been used to detect Feridex (dextran-coated superparamagnetic iron oxide [SPIO]-labeled rat, porcine, and human islets transplanted into rodents).<sup>5–7</sup> In the present study, we used a novel MRI contrast agent, chitosan-coated SPIO (CSPIO) nanoparticles,<sup>8</sup> to monitor mouse islet grafts.

From the Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung University and Chang Gung Memorial Hospital (J.-H.J., Y.-W.C.), Department of Medical Imaging and Radiologic Sciences (J.-J.W.), and Department and Graduate Institute of Medical Biotechnology and Laboratory Science (C.-R.S., H.-Y.K.), Chang Gung University, Taoyuan, the Molecular Imaging Center, Chang Gung Memorial Hospital, Taoyuan (C.-R.S., Z.-T.T., T.-C.Y.), and the Department of Biologic Science and Technology, National Chiao Tung University, Hsinchu (C.-H.K.), Taiwan.

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Address reprint requests to Dr Juahn-Huarng Juang, Division of Endocrinology and Metabolism, Chang Gung Memorial Hospital, 5 Fu-Shin Street, Kweishan, Taoyuan, Taiwan. E-mail: jjuang@adm.cgmh.org.tw

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## MATERIALS AND METHODS

## Animals

Male inbred C57BL/6 mice, aged 8–12 weeks, were used as donors and recipients of islet transplantation.<sup>9</sup> The animal experiments were approved by the Animal Ethics Committee of Chang Gung Memorial Hospital.

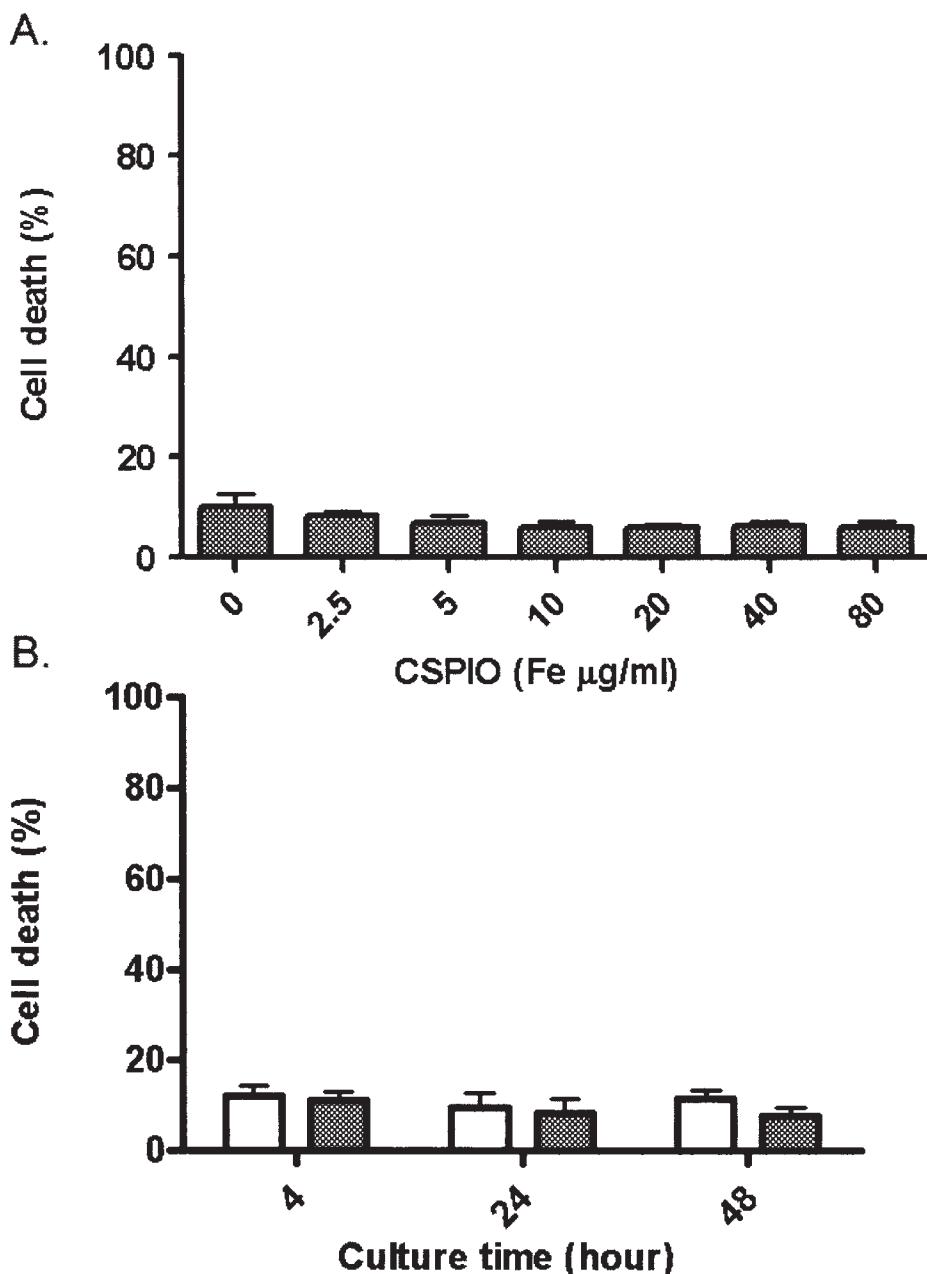
## Islet Isolation

Under anesthesia with sodium amobarbital, pancreases were distended with 2.5 mL RPMI-1640 medium (Gibco, Grand Island,

NY, USA) containing 1.5 mg/mL collagenase (collagenase from *Clostridium histolyticum*, type XI; Sigma Immunochemicals, St Louis, Mo), excised and incubated in a water bath at 37°C.<sup>9</sup> The islets were separated by a density gradient (Histopaque-1077; Sigma Immunochemicals), and purified islets were then hand-picked under a dissecting microscope.

## Islet Labeling

Isolated islets were overnight incubated with CSPIO (10 mg/mL) in culture medium.<sup>8</sup> After incubation, islets were washed with culture medium and used for in vitro studies or islet transplantation.



**Fig 1.** Cytotoxicity of chitosan-coated superparamagnetic iron oxide (CSPIO) on RAW cells. RAW cells were incubated with CSPIO of different concentrations (A) and different time periods (B; open columns: control; shaded columns: Fe 20  $\mu\text{g}/\text{mL}$ ) and then stained with fluorescein diacetate and propidium iodide. Cell death rates were assayed by flow cytometry analysis.

### Islet Cytotoxicity

RAW cells were incubated with CSPIO and then stained with fluorescein diacetate and propidium iodide. Cell viability and death rate were assayed by flow cytometry analysis.<sup>10</sup>

### Islet Transplantation

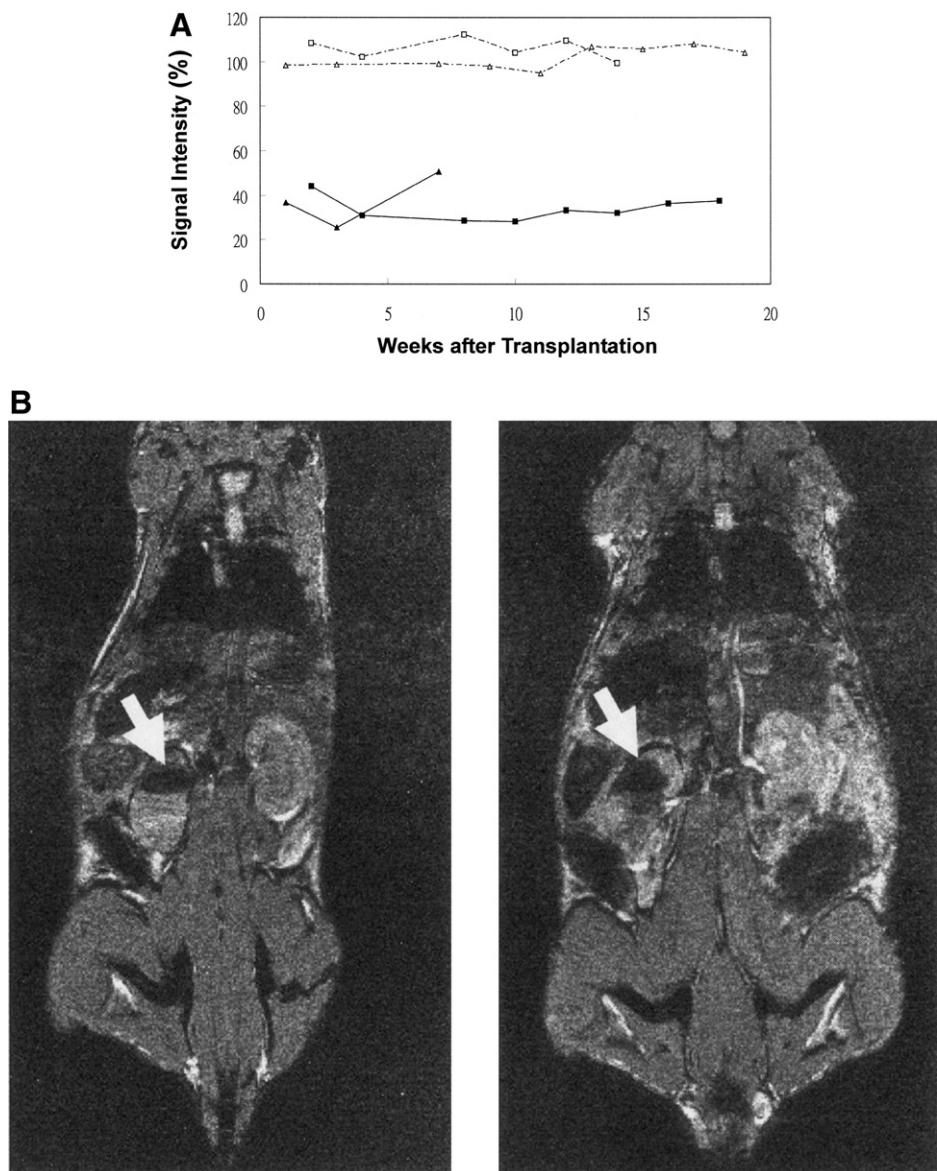
Three hundred islets cultured with and without CSPIO were syngeneically transplanted under the left kidney capsule of each mouse.<sup>9</sup> To accomplish this, the islets were centrifuged in PE-50 tubing (Clay Adams, Parsippany, NJ) connected to a 200- $\mu$ L pipette tip. With the mouse under amobarbital anesthesia, the left kidney was exposed through a lumbar incision. A capsulotomy was performed in the lower pole of the kidney and the tip of the tubing advanced under the capsule of the upper pole, the site of final injection. The capsulotomy was left unsutured.

### In Vivo MRI of Transplanted Islets

After transplantation, serial MRI of the recipients (2 with control islets and 2 with CSPIO-labeled islets) were performed biweekly. One control animal and 1 CSPIO-labeled animal died at 9 and 16 weeks, respectively. The other control animal and 1 CSPIO-labeled animal were followed until 19 and 18 weeks, respectively. Images were acquired on a 3.0-Tesla MRI scanner (Magnetom Trio with TIM; Siemens, Erlangen, Germany) using a homemade surface coil. A T2\*-weighted gradient-recalled echo sequence was acquired for all subjects. The MR signal intensity of the islet graft was quantified using the contralateral kidney as a reference.

### Removal of the Islet Graft

Eighteen weeks after transplantation, animals intended for graft removal were anesthetized with amobarbital. An abdominal incision was made and the kidney was exposed. Under dissecting



**Fig 2.** **A.** Evolution of the magnetic resonance (MR) signal intensity of islet isografts in controls (open squares and triangles) and animals with chitosan-coated superparamagnetic iron oxide (CSPIO)-labeled islets (solid squares and triangles). The MR signal intensity of the contralateral kidney was used as a reference. **B.** The 2-week (left) and 18-week (right) MR image of the CSPIO-labeled islet graft showing a distinct hypointense spot homogeneously located at the upper pole of left kidney (arrows).

microscope, the kidney capsule surrounding the graft was excised and removed with the adherent graft.<sup>11</sup>

#### Immunohistochemistry of the Islet Graft

The removed grafts were fixed in formalin solution and processed for paraffin embedding and sectioning. Sections of grafts were stained for iron with Prussian blue and for the endocrine  $\beta$ -cells with a guinea pig anti-swine insulin antibody (Dako Co., Glostrup, Denmark).<sup>11</sup>

#### RESULTS

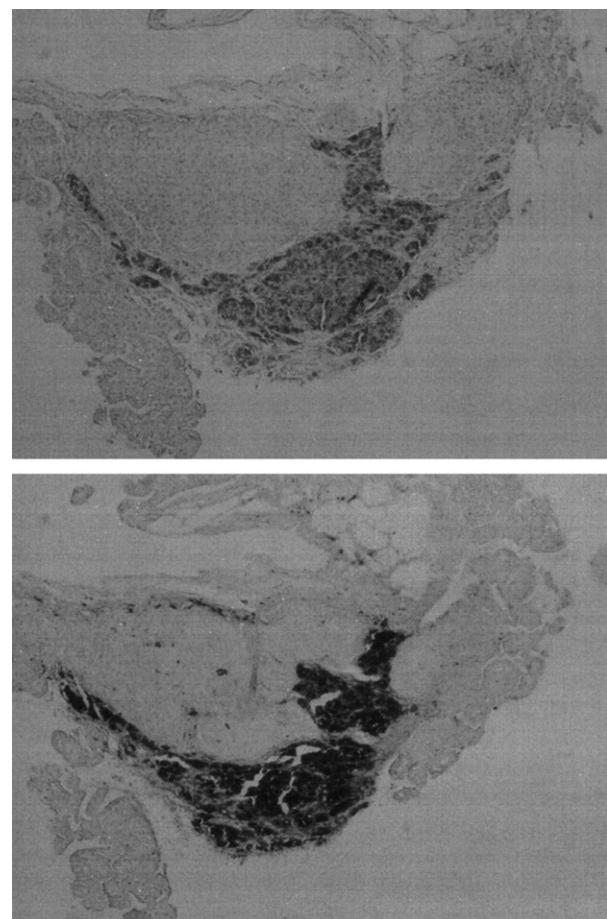
After incubation with CSPIO, the cell death rates in RAW cells did not increase with increasing CSPIO concentrations or incubation time (Fig 1). In contrast to the control, the grafts of CSPIO-labeled islets were visualized on MRI scans as distinct hypointense spots homogeneously located at the upper pole of the left kidney. There was a 30%–50% signal loss throughout the follow-up period (Fig 2). At 18 weeks, the CSPIO-labeled islet graft was positive for insulin and iron staining and the insulin- and iron-stained areas were almost identical (Fig 3).

#### DISCUSSION

An MRI technique has been used to detect Feridex-labeled rat, porcine, and human islets transplanted into rodents.<sup>5–7</sup> Dextran-coated Feridex is approved by the US Food and Drug Administration for human use as a liver imaging contrast agent. Unfortunately, in November 2008 the company ceased to manufacture Feridex. Recently, Tsai et al. developed a novel MRI contrast agent, CSPIO, by coating SPIO with chitosan to increase the content of magnetite.<sup>8</sup> In the present study, we demonstrated that isolated mouse islets labeled with CSPIO nanoparticles could be visualized by MRI after transplantation.

Chitosan has been applied in numerous biomedical applications owing to its nontoxicity, biocompatibility, and biodegradability.<sup>12</sup> Here, we showed that the cell death rates in RAW cells did not increase with increasing CSPIO concentrations or incubation time. This indicates that CSPIO can be safely used to label cells for MRI tracking.

On MRI scans, the iron oxide leads to an extensive signal loss owing to magnetic field inhomogeneity. Therefore, the grafts of Feridex-labeled<sup>5–7</sup> and CSPIO-labeled islets showed distinct hypointense spots homogeneously located at the transplantation sites. In the present study, we have for the first time quantified the graft MR signal intensity by using the contralateral kidney as a reference. The MR signal intensity of CSPIO-labeled islet grafts was 30%–50% that of control grafts. Moreover, this signal loss was maintained for 18 weeks, indicating the persistent existence of CSPIO at the transplantation site. To our knowledge, this is the longest MRI follow-up for SPIO-labeled islet grafts. At 18 weeks, the graft histology revealed that the insulin- and iron-stained areas were almost identical. This further supports the notion that the uptake of CSPIO by isolated islets leads islet grafts to be visualized by MRI after transplanta-



**Fig 3.** The chitosan-coated superparamagnetic iron oxide-labeled islet graft stained with insulin (top, brown color) and Prussian blue (bottom, blue color) at 18 weeks after islet transplantation.

tion. In conclusion, isolated mouse islets labeled with CSPIO nanoparticles can be effectively and safely imaged by using MRI as long as 18 weeks after transplantation.

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